

# Electric-Field-Driven Transformations of a Supported Model Biological Membrane

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Phospholipid bilayers (films), formed by fusing small unilamellar vesicles (SUVs) onto surfaces, constitute an attractive model of a biological membrane used to study cell-membrane processes.<sup>1</sup> These membranes can also be used to study the influence of an electric field on voltage-gated membrane proteins, lipid-lipid interactions, and lipid-protein interactions.<sup>2</sup> In addition, films of phospholipids with incorporated proteins deposited on a metal or metal-oxide electrode have important applications in the development of novel electrochemical sensors.<sup>3</sup> At an electrode surface, phospholipid films are exposed to high electric fields on the order of  $10^9$  V/m. A field of this magnitude affects the membrane properties, and the knowledge of this effect is needed for both scientific and practical applications of supported phospholipid bilayers.

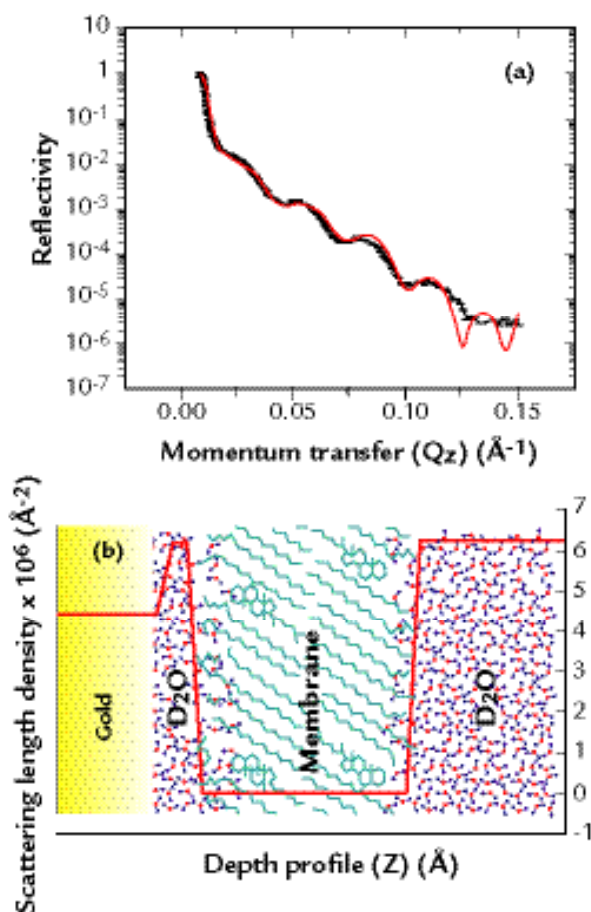
## Surface Profile Analysis Reflectometer Experiment

As a model biological membrane, we used a mixed dimyristoylphosphatidylcholine (DMPC)/cholesterol bilayer (70:30 mol % ratio) deposited on a gold electrode surface by vesicle fusion. Because DMPC is a neutral (zwitterionic) lipid, the field acting on this membrane is solely due to the charge on the metal. Our electrochemical studies show that the membrane is stable at the electrode surface in the charge-density range of  $\pm 8 \mu\text{C cm}^{-2}$  (from -450 mV to +350 mV versus Ag/AgCl, which is the standard reference used by electrochemists for measuring potentials). Above or below these values, the charge-density curve for the membrane-covered electrode merges with the curve for the film-free interface. The merging of these two curves indicates that the membrane has become detached from the gold surface.

We have performed neutron-scattering experiments using the time-of-flight Surface Profile Analysis Reflectometer (SPEAR) at the Los Alamos Neutron Science Center (LANSCE) to investigate electric-field-driven changes of the membrane structure. Due to scattering-length-density (SLD) differences between gold,  $\text{CH}_2$ -chains, and  $\text{D}_2\text{O}$ , the thickness of the membrane and its water content ( $\text{D}_2\text{O}$ ) can be determined from SLD curves.

## Results

We have demonstrated that a model biological membrane is stable when the charge on the metal is less than  $-8 \mu\text{C cm}^{-2}$  or when the field acting at the membrane is less than  $5 \times 10^9$  V/m. At zero charge on the metal, the DMPC chains are tilted, and the membrane contains defects.



By charging the metal and increasing the field, the chains become more porous and less tilted. At a potential of -750 mV versus Ag/AgCl (i.e., corresponding to a surface charge of  $-23 \mu\text{C cm}^{-2}$  and a field exceeding  $5 \times 10^9 \text{ V/m}$ ), the membrane becomes detached from the electrode. We have shown for the first time that this membrane remains in close proximity to the metal electrode and is suspended on a thin cushion of the electrolyte (Fig. 1). This membrane is essentially defect free. Additionally, the charge-driven changes in the membrane structure are fully reversible. By turning a knob on a control instrument, one can inject or withdraw the charge from the metal surface. The membrane responds to these changes either by being lifted up or by being directly deposited onto the metal. This ability to use the electric field to control the membrane structure opens new opportunities for biomimetic research.

**Fig. 1.** (a) Neutron-reflectivity data as a function of the momentum transfer vector  $Q_z$  (in  $\text{\AA}^{-1}$ ) obtained for a mixed cholesterol/DMPC bilayer spread at the electrode surface when the surface charge density of the gold is highly negative (i.e.,  $-23 \mu\text{C cm}^{-2}$  corresponding to -750 mV versus Ag/AgCl). The red line is the fit resulting from a one-layer model used to represent the biomimetic film. It is important to note that even though the film is now no longer directly attached to the gold electrode, neutron reflectivity still detects its presence in close vicinity to the support. This is a unique ability of the neutron-reflectivity technique. (b) Profile of the biomimetic film/gold interface in the direction perpendicular to the electrode. The red line is the SLD profile, determined from the neutron-reflectivity curve given above in (a). The model shows a defect-free biological film with no solvent penetration cushioned by a thin film of solvent.

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